

RayBio[®] Human Protein Array G Series

Glass–chip-based protein arrays

User Manual (Revised Dec. 3, 2007)

For detecting protein–protein interactions, antibody specificity, auto-antibody, protein modifications and small molecule–protein interactions

RayBio[®] Human Protein Array G Series 1 (Cat# PAH-G1)
RayBio[®] Custom Human Protein Array G Series (Cat# PAH-CUST-G)
RayBio[®] Human Protein Array G Series Service (Cat# PAH-SERV-G)



**We Provide You with Excellent
Protein Array System and Service**

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RayBiotech, Inc.

RayBio[®] Human Protein Array G Series Protocol

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RayBio[®] is the trademark of RayBiotech, Inc.

I. Introduction

RayBio[®] Protein Arrays are a series of products developed by RayBiotech, Inc., The Protein Array Pioneer Company. Recombinant or native proteins are spotted onto the surface of a solid support, such as membrane or glass slide. The kits can be applied in screening protein-protein interactions, monitoring auto-antibody expression, determining antibody specificity, identifying protein modifications and detecting small molecule-protein interactions.

Custom protein arrays are also available from RayBiotech, Inc. You can select your own proteins of interest or provide your own proteins. RayBiotech, Inc., then produces custom protein arrays for you.

Applications

Since RayBio[®] protein arrays have multiple applications, which require different procedures, only several examples are given here.

1. Detection of protein-protein interactions. The kit can be used to screen novel protein-protein interactions, validate the previously known protein-protein interactions and examine the interaction conditions.
2. Characterization of antibodies. The kit can be used to test the specificity of an antibody.
3. Target identification. The kit can be used to screen the small molecule-protein interaction for target identification, drug discovery and toxicity study.
4. Detection of autoantibodies. The kit can be used to characterize autoantibodies from body fluids.
5. Detection of protein modifications. The kit can also be used to determine protein modifications such as phosphorylation.
6. Detection of protein-DNA interaction. In some cases, the kit can be used to detect DNA binding protein.

Features of RayBio[®] Protein Arrays

- * High-throughput approach to detect multiple protein functions, including protein-protein interactions, protein modifications, antibody specificity, auto-antibody expression and small molecule-protein interactions.
- * Both glass and membrane format.
- * Affordable, quick and simple to use.
- * No equipment required if membrane-based arrays are used.

II. Materials Provided

Upon receipt, all components of the RayBio[®] Human Protein Array kit should be stored at -20°C to -80°C until just before the experiment. At -20°C to -80°C the kit will retain complete activity for up to 6 months. Please use within six months of purchase. Once thawed, the array glass chip and Blocking Buffer should be kept at -20°C and all other component should be stored at 4°C. Use within three months after reagents have been thawed.

For glass chip-based protein arrays

RayBio[®] Human Protein Array Chip (2, 4, or 8 arrays)
1,000 X Biotin-conjugated anti-IgG
(1.5 µl/vial of Biotin-conjugated anti-mouse IgG,
1.5 µl/vial of Biotin-conjugated anti-rabbit IgG,
1.5 µl/vial of Biotin-conjugated anti-human IgG)
2 X Blocking Buffer (4 ml)
20 X Wash Buffer I (15 ml)
20 X Wash Buffer II (15 ml)
2 X Binding Buffer (RIPA buffer, 20 mM Tris, pH 7.5, 0.3 M NaCl, 2% sodium deoxycholate, 2% TX-100) 10 ml
1,000 X Alexa Fluor 555-Conjugated Streptavidin (1.5 µl)

Additional Materials Required

Depending on your specific purpose, different additional materials are needed, such as:

- Orbital shaker
- Laser scanner for fluorescence detection
- Aluminum foil
- Distilled water
- Plastic box
- Binding buffer of your own

III. Overview and General Considerations

A. Preparation of Samples

Depending on your experimental purpose, different samples may be used. To detect protein-protein interaction, you may need to label your protein with biotin or use an antibody against input protein. To profile auto-antibodies, you need to prepare serum or plasma. To characterize the specificity of antibody, you need have the antibody of your interest.

Optimization of experimental conditions: *If you experience high background, you may need to further dilute your sample and/or wash slides in Wash Buffer I overnight at 4°C. If the signal is too weak, you may increase the amount of your sample and/or increase incubation times of one or more steps.*

B. Handling glass chips

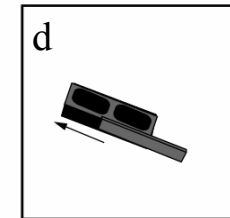
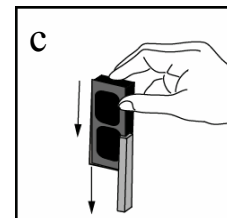
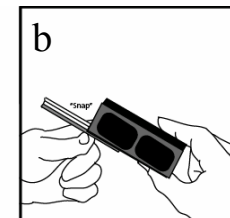
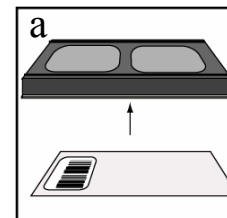
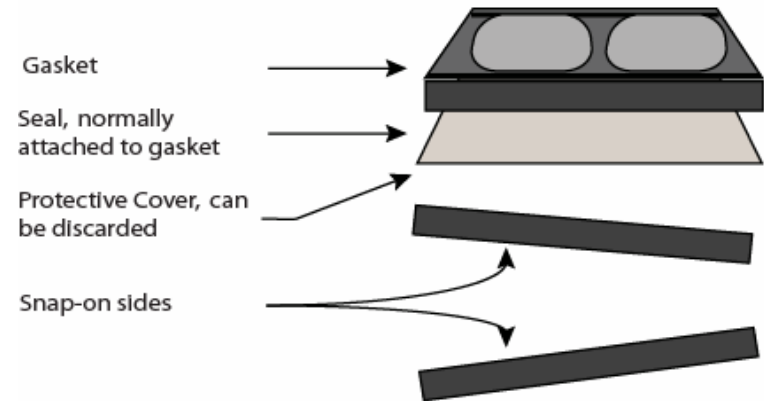
- The microarray slides are sensitive. Do not touch the surface. Hold the slides by the edges only.
- Handle all buffers and slides with powder-free gloves.
- Avoid breaking glass slide.
- Handle glass chip in clean environment.

C. Incubation

- Completely cover array area with sample or buffer during incubation, and cover the incubation chamber with adhesive film or plastic sheet protector to avoid drying.
- Avoid foaming during incubation steps.
- Perform all incubation and wash steps under gentle rotation or shaking.
- Cover the incubation chamber with adhesive film during incubation, particularly when incubation is more than 2 hours or less than 400 µl of sample or reagent is used.
- Avoid cross-contamination from overflowing solution to neighboring wells.
- Several incubation steps such as blocking, sample incubation, biotin-Ab incubation or Alexa Flour 555-streptavidin incubation may be done at 4°C for overnight. Please make sure to cover the incubation chamber tightly to prevent evaporation.

D. Incubation chamber assembly

Instructions for gasket assembly L series arrays



a. Apply slide to incubation chamber barcode facing upward as in step (a).

b. Gently snap one edge of a snap-on side as shown in step (b).

c. Gently press other of side against lab bench and push in direction shown in step (c).

d. Repeat with the other side.

IV. Protocol

A. Detection of protein-protein interaction

Several strategies can be used for detection of protein-protein interaction as shown in the following figure 1.

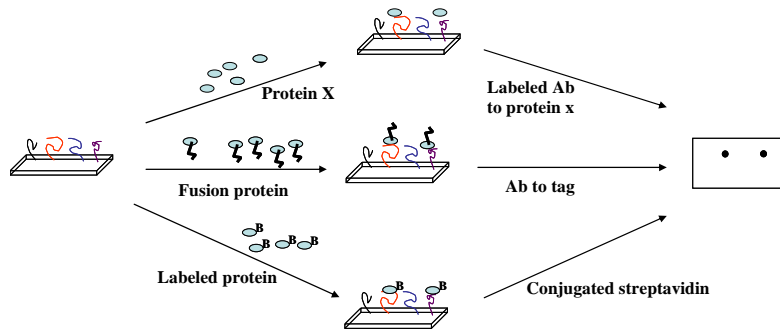


Fig. 1. Three common strategies for detection of protein-protein interaction using RayBio® protein array kits.

RayBiotech can assist you in your experiment design and provide testing services for your project. Please feel free to contact us.

The following protocol is for use of antibody against the probe protein.

a. Blocking and Incubation

Note: if your slide has been assembled, you can go to step 3 directly.

1. Take the glass chip out from the box. Let air dry for at least 60 minutes in a laminar flow hood.
2. Assemble the glass chip into incubation chamber and incubation frame as shown in Section III D.
3. Dilute 2x Blocking Buffer with H₂O. Add 500 µl 1x Blocking Buffer into each well and incubate at room temperature for 30 min to block slides. Make sure no bubbles are in the well.

Note: only add reagents to wells printed with proteins.

4. Decant Blocking Buffer from each well. Add 400 µl of each sample to each well. Incubate arrays with sample at room temperature for 1 to 2 hours or other condition as appropriate.

Note: We recommend using 1 to 100 µg of total protein in your experiment. Adjust the amount of protein accordingly if background is high, use less amount of protein. If the signals are weak, use more protein. Different protein-protein interactions may need different binding buffers.

5. Dilute 20X Wash Buffer I with H₂O. Decant the samples from each well, and wash 5 times with 800 µl of 1X Wash Buffer I at room temperature with gentle shaking 2 min per wash. Completely remove wash buffer I in each wash step.

Note: avoid solution flowing into neighboring wells.

6. Dilute 20X Wash Buffer II with H₂O. Wash 2 times with 800 µl of 1X Wash Buffer II at room temperature with shaking. 2 min per wash. Completely remove wash buffer II in each wash step.

b. Detection of associated protein

Depending on the different strategies in the experimental design, different protocols can be used.

If the biotin labeled-protein is used as the probe, follow this step.

1. Dilute 1,000x Alexa Fluor 555 in H₂O. Add 400 µl of 1 X Alexa Fluor 555-conjugated streptavidin (after brief spinning, add 1.5 ml of 1 X Blocking Buffer to Alexa Fluor 555-conjugated streptavidin tube) to each subarray. Cover the incubation chamber with Adhesive film. Cover the plate with aluminum foil to avoid exposure to light or incubate in dark room.
 2. Incubate at room temperature for 1 to 2 hours.
- Note: incubation may be done at 4°C for overnight.*
3. Wash with Wash Buffer I as directed in steps a.5 and Wash Buffer II as described in step a.6.

If non-labeled protein is used, antibody against the protein or fusion tag must be available.

1. Add 400 µl of appropriate diluted antibody into each well. Incubate at room temperature for 2 hours.

Note: incubation may be done at 4°C for overnight. Usually 1 ng/ml to 1,000 ng/ml of antibody will be used. You will need to optimize the dilution factor needed for your particular antibody in this experiment.

- Wash with Wash Buffer I as directed in steps 5 and Wash Buffer II as described in step 6.
- Add 400 μ l of 1,000-fold diluted biotin labeled anti-IgG (Choose anti-mouse IgG for mouse antibody, anti-rabbit for rabbit antibody, etc). Incubate at room temperature for 1 hour.
- Wash with Wash Buffer I as directed in steps 5 and Wash Buffer II as described in step 6.
- Add 400 μ l of 1X Alexa Flour-555 Streptavidin (after brief spinning, add 1.5 ml of Blocking Buffer to Alexa Flour 555-conjugated streptavidin tube) to each subarray. Cover the incubation chamber with Adhesive film. Cover the plate with aluminum foil to avoid exposure to light or incubate in dark room.
- Incubate at room temperature for 1 to 2 hour.
- Wash with Wash Buffer I as directed in steps a.5 and Wash Buffer II as described in step a.6.

c. Fluorescence Detection

- Decant excess Wash Buffer from wells.
- Disassemble the slide out of the incubation frame and chamber.
- Place the whole slide in a 50 ml centrifuge tube. Add enough Wash Buffer I (about 30 ml) to cover the whole slide and gently shake or rock at room temperature for 10 minutes. Decant Wash Buffer I. Repeat with Wash Buffer I once. Wash with Wash Buffer II (about 30 ml) with gentle shake at room temperature for 10 minutes. Or wash using slide chamber. Rinse the slide with distilled H₂O.
- Remove water droplets by centrifuge at 1,000 rpm for 3 minutes and then let slide dry completely in air at least 20 minutes (protect from light). Make sure the slides are absolutely dry before starting the scanning procedure.
- Image the signals using laser scanner (such as Axon GenePix) using cy3 (green) channel.

Note: Although we recommend scanning slides right after experiment, you also can store the slide at -20°C in dark for several days. If you do not have a laser scanner, send your slide to us and we can scan it for you.

B. Characterization of antibody specificity.

Several strategies can be used for detection of antibody specificity as shown in the following figure 2.

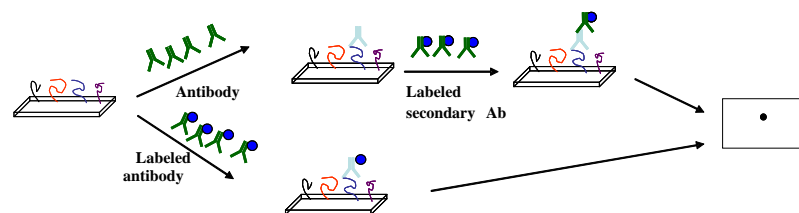


Fig. 2. Determination of antibody specificity using RayBio® protein array kits.

RayBiotech can assist you in your experiment design and provide testing services for your project. Please feel free to contact us.

The following protocol is for use of labeled secondary antibody

a. Blocking and Incubation

Note: if you slide has been assembled, you can go to step 3 directly.

- Take the glass chip out from the box. Let air dry for 60 minutes in laminar flow hood.
- Assemble the glass chip as described in Section III D.
- Add 500 μ l of 1 X Blocking Buffer into each well and incubate at room temperature for 30 min to block slides. Dilute 2X Blocking Buffer with H₂O. Make sure no bubbles are in the well.
- Remove blocking buffer from well. Add 400 μ l of sample such as anti-serum or IgG. Incubate at room temperature for 1 hour.
- Decant the samples from each well, and wash 5 times with 800 μ l of 1X Wash Buffer I at room temperature with gentle shaking. 2 min per wash. Dilute 20X Wash Buffer I with H₂O. Completely remove wash buffer I in each wash step.

Note: avoid solution flowing into neighboring wells.

- Wash 2 times with 800 μ l of 1X Wash Buffer II at room temperature with shaking. 2 min per wash. To prepare 1X Wash buffer, dilute 20X

Wash Buffer II with H₂O. Completely remove wash buffer II in each wash step.

7. Add 400 µl of 1,000 fold diluted Biotin label-secondary antibody (choose appropriate secondary antibody, use biotin label-anti-mouse IgG for mouse antibody; use biotin label-anti-rabbit IgG for rabbit antibody). To prepare 1X Biotin-labeled secondary antibody, add 1.5 ml of blocking buffer to biotin label-secondary antibody tube.
8. Wash with Wash Buffer I as directed in steps 6 and Wash Buffer II as described in step 7.

b. Fluorescence Detection

1. Decant excess Wash Buffer from wells.
2. Disassemble the slide out of the incubation frame and chamber.
3. Place the whole slide in a 50 ml centrifuge tube. Add enough Wash Buffer I (about 30 ml) to cover the whole slide and gently shake at room temperature for 10 minutes. Decant Wash Buffer I. Repeat once with Wash Buffer I. Wash with Wash Buffer II (about 30 ml) with gentle shake at room temperature for 10 minutes. Or wash using slide chamber. Rinse the slide with distilled H₂O.
4. Remove water droplets by centrifuge at 1,000 rpm for 3 minutes and then let slide dry completely in air at least 20 minutes (protect from light). Make sure the slides are absolutely dry before the scanning procedure.
5. Image the signals using laser scanner (such as Axon GenePix) using cy3 (green) channel.

Note: Although we recommend scanning slides right after experiment, you also can store the slide at -20°C in dark for several days. If you do not have a laser scanner, RayBiotech can provide service for you. Just simply send your slide to us and we will take care of it.

C. Detection of auto-antibody.

The following strategy can be used for detection of autoantibody as shown in the following figure 3.

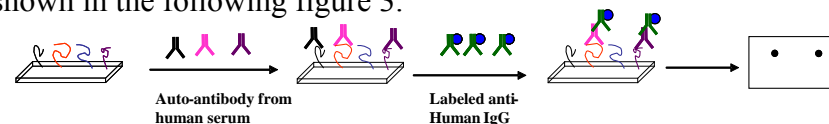


Fig. 3. Identification of autoantibody using RayBio® protein array kits.

RayBiotech can assist you in your experimental design and provide testing services for your project. Please feel free to contact us.

a. Blocking and Incubation

Note: if your slide has been assembled, you can go to step 3 directly.

1. Take the glass chip out from the box. Let air dry for at least 60 minutes.
2. Assemble the glass chip as described in section III D.
3. Add 500 µl of 1 X Blocking Buffer into each well and incubate at room temperature for 30 min to block slides. Dilute 2X Blocking Buffer with H₂O. Make sure no bubbles are in the well.
4. Add 400 µl of serum or plasma or other body fluids. Incubate at room temperature for 1 hour. Dilute serum or plasma samples 5 to 50 fold.

Note: Since auto-antibody concentrations in serum and plasma may vary widely, you may need to optimize this dilution for your samples.

5. Decant the samples from each well, and wash 5 times with 800 µl of 1X Wash Buffer I at room temperature with gentle shaking. 2 min per wash. Dilute 20X Wash Buffer I with H₂O. Completely remove wash buffer I in each wash step.

Note: avoid solution flowing into neighboring wells.

6. Wash 2 times with 800 µl of 1X Wash Buffer II at room temperature with shaking. 2 min per wash. Dilute 20X Wash Buffer II with H₂O. Completely remove wash buffer II in each wash step.
7. Add 400 µl of 1,000-fold diluted biotinylated anti-human IgG (after brief spinning, add 1.5 ml of block buffer to the biotin anti-human IgG vial). Incubate at room temperature for 1 to 2 hours.

8. Wash as steps 5 and 6.
9. Add 400 μ l of 1X Alexa Flour-555 Streptavidin (after brief spinning, add 1.5 ml of Blocking Buffer to Alexa Flour 555-conjugated streptavidin tube) to each subarray. Cover the incubation chamber with Adhesive film. Cover the plate with aluminum foil to avoid exposure to light or incubate in dark room.
10. Incubate at room temperature for 1 to 2 hours.

Note: incubation may be done at 4°C for overnight.

11. Wash with Wash Buffer I as directed in steps 7 and Wash Buffer II as described in step 8.

b. Fluorescence Detection

1. Decant excess Wash Buffer from wells.
2. Disassemble the slide out of the incubation frame and chamber.
3. Place the whole slide in a 50 ml centrifuge tube. Add enough Wash Buffer I (about 30 ml) to cover the whole slide and gently shake at room temperature for 10 minutes. Decant Wash Buffer I. Repeat Wash Buffer I once. Wash with Wash Buffer II (about 30 ml) with gentle shake at room temperature for 10 minutes. Or wash using slide chamber. Rinse the slide with distilled H₂O.
4. Remove water droplets by centrifuge at 1,000 rpm for 3 minutes and then let slide dry completely in air at least 20 minutes (protect from light). Make sure the slides are absolutely dry before the scanning procedure.
5. Image the signals using laser scanner such as Axon GenePix using cy3 channel.

Note: we recommend scanning slides right after experiment. You also can store the slide at -20°C in dark for several days. If you do not have a laser scanner, RayBiotech can provide service for you. Just simply send your slide to us and we will take care of it.

D. Detection of small molecule-protein interaction

Several strategies can be used for detection of small molecule-protein interaction as shown in the following figure 4.

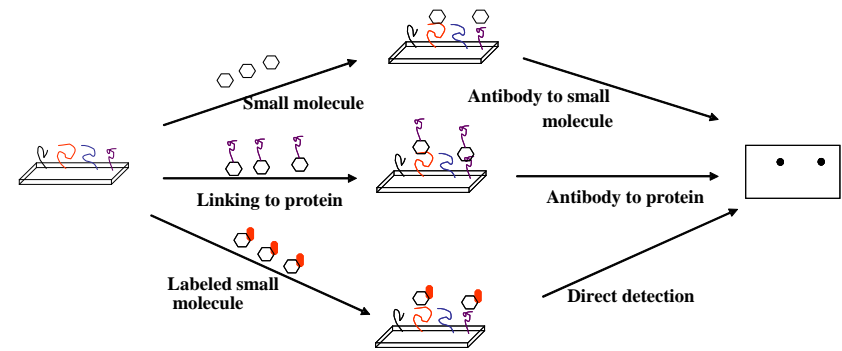


Fig. 4. Detection of small molecule-protein interaction using RayBio® protein array kits.

RayBiotech can assist you in your experimental design and provide service for your project. Please contact us.

E. Detection of protein modification

RayBio® Human Protein Arrays may also be used to detect protein modification. Several strategies to detect phosphorylation modification are shown in the following figure 5.

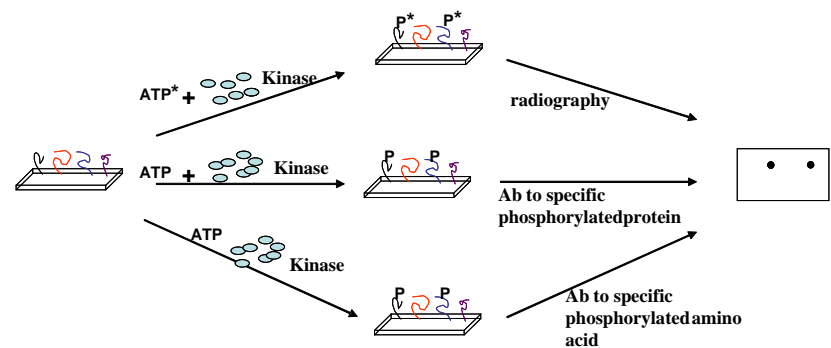


Fig. 5. Three common strategies for detection of protein modification using protein array kits.

RayBiotech can assist you in your experiment design and provide service for your project. Please contact us.

V. Interpretation of Results:

The following figure shows RayBio® Human Protein Array 1 probed with different patients' serum. The images were captured using laser scanner. The biotin-conjugated protein produces positive signals, which can be used to identify the orientation and to compare the relative expression levels among the different wells.

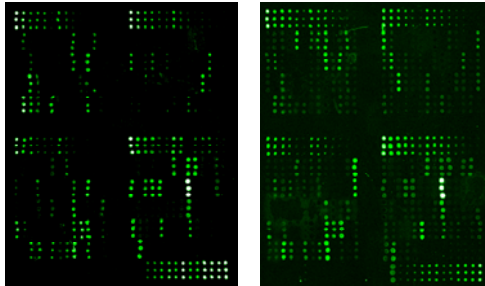


Fig. 6. Auto-antibody profiles from two different patients' sera were determined using RayBio® Human Protein Array 1.

The signal intensities obtained from laser scanner can simply be imported into our analysis tool. The analysis tool will help you:

- Locate your signal intensities to protein array map
- Protein list sorting
- Average signal intensities
- Subtract background
- Normalize the data from different samples
- Obtain comparison charts among different samples

This analysis tool is very simple and affordable, which will not only assist in compiling and organizing your data, but also reduces your calculations to a “copy and paste” step.

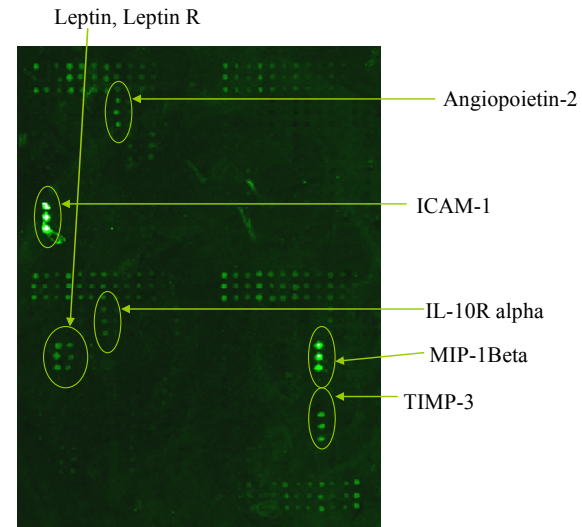


Fig. 7. Determine the leptin-associated protein using RayBio® Human Protein Array 1. Human protein array 1 was incubated with recombinant leptin for 2 hours, followed by incubation with anti-leptin antibody. The data clearly indicates that leptin binds to several proteins in addition to leptin receptor.

Caution for interpretation of results

1. The *in vitro* and *in vivo* protein function may behave differentially.
2. Some recombinant proteins contain a tag sequence. Some recombinant proteins lack certain domain of protein, particularly hydrophobic domain. The modification status of those proteins is largely unknown. All these may affect the protein-protein interaction.
3. Almost all membrane-bound proteins only contain extracellular domain, but lack transmembrane and cytoplasmic domains.
4. Different proteins may require distinct conditions for their optimal function. Therefore, investigators in some case may need to use different conditions for array experiment.
5. Always perform control experiment since both IgG or streptavidin may bind to some proteins.

RayBio® Human Protein Array 1 List

1 4-IBBL	51 Eotaxin-3	101 IL-2R alpha	151 MEC (CCL-28 / VIC)	201 TARC
2 4-IBBR	52 ErbB3	102 IL-2R beta	152 MICA	202 TECK
3 Acrp30	53 Erythropoietin R	103 IL-2R gamma	153 MICB	203 TGF-a
4 Activin A	54 E-selectin	104 IL-3	154 MIF	204 TGF-beta 1
5 ALCAM	55 Fas	105 IL-4	155 MIG	205 TGF-beta 2
6 Angiogenin	56 Fas Ligand	106 IL-5	156 MIP-1a	206 TGF-beta 3
7 Angiopoietin-1	57 Fc Gamma RII B/C (CD32b/c)	107 IL-6	157 MIP-1b	207 Tie-1
8 Angiopoietin-2	58 FGF-4	108 IL-6	158 MIP-1d	208 Tie-2
9 Angiostatin	59 FGF-6	109 IL-6 sR	159 MIP-3a	209 Timp-1
10 AR (Amphiregulin)	60 FGF-7	110 IL-7	160 MIP-3b	210 Timp-2
11 ART	61 FGF-9	111 IL-8	161 MMP-1	211 Timp-3
12 AXL	62 FGF-b	112 IL-9	162 MMP-2	212 Timp-4
13 b NGF	63 Fcγ3 Ligand	113 IL-9 R	163 MMP-3	213 TNF-a
14 B7-1 (CD 80)	64 Follistatin	114 IL-10	164 MMP-8	214 TNF-b
15 BCAM	65 Fractalkine	115 IL-10 R alpha	165 MMP-9	215 TNF-R I
16 BDNF	66 GCP-2	116 IL-10 R beta	166 MMP-10	216 TNF-R II
17 BLC	67 GCSF	117 IL-11	167 MMP-13	217 TPO
18 BMP-4	68 GDNF	118 IL-12p40	168 MPlf-1	218 TRAIL
19 BMP-5	69 GH	119 IL-12p70	169 MSP	219 TRAIL R1
20 BMP-6	70 GHR	120 IL-13	170 NAP-2	220 TRAIL R2
21 BMP-7	71 GHR Ligand	121 IL-13 Ra1	171 NGF R	221 TRAIL R3
22 BTC	72 GLP-1 (Glucagon Like)	122 IL-13 Ra2	172 NRG-1b1	222 TRAIL R4
23 Cardiotrophin-1 (CT-1)	73 GM-CSF	123 IL-15	173 NT-3	223 TRANCE
24 Cathepsin S	74 gp130	124 IL-16	174 NT-4	224 TREM-1
25 CCL21 / 6CKine	75 GRO-a	125 IL-17	175 OBS (Obstatin-C-terminus)	225 TROY
26 CD14	76 GRO-b	126 IL-17B	176 Oncostatin M (OSM)	226 u PAR
27 CD27	77 GRO-r	127 IL-17C	177 Osteoprotegerin (OPG)	227 ubiquitin+1
28 CD30	78 HB-EGF	128 IL-18 BPa	178 PARC	228 VCAM-1
29 CD40	79 HCC-4	129 IL-18 R alpha	179 P-Cadherin	229 VE- Cadherin
30 CD40 Ligand	80 HGF	130 IL-18 R beta	180 PDGF AA	230 VEGF
31 CK beta 8-1 / CCL23	81 HVEM	131 IL-21R	181 PDGF AB	231 VEGF R2
32 CNTF	82 I-309	132 IL-28A / IFN-lambda	182 PDGF BB	232 VEGF R3
33 Complement Factor D	83 ICAM-1	133 Insulin	183 PDGF R alpha	233 VEGF-D
34 C-peptide	84 ICAM-2	134 Insulin-Receptor	184 PDGF R beta	234 VEGI
35 CTACK	85 ICAM-3	135 IP-10	185 PECAM-1 (CD31)	
36 CTLA-4	86 IFN-gamma	136 I-TAC	186 PF-4	
37 CXCL-16	87 IGF-1R	137 LAP	187 PIGF	
38 DAN	88 IGFBP-1	138 Leptin (OB)	188 Prolactin	
39 DKK-1	89 IGFBP-2	139 Leptin R	189 P-selectin	
40 DKK-4	90 IGFBP-3	140 LIF	190 RAGE	
41 DR 6	91 IGFBP-4	141 Light	191 Rantes	
42 Dkk	92 IGFBP-6	142 L-selectin	192 SAA	
43 E-Cadherin	93 IGF-1	143 Lymphotactin	193 SCF	
44 EGF	94 IGF-II	144 MCP-1	194 SCF R	
45 EGF-R	95 IL-1a	145 MCP-2	195 SDF-1a	
46 ENA-78	96 IL-1b	146 MCP-3	196 SDF-1b	
47 Endoglin	97 IL-1ra	147 MCP-4	197 Shh-N	
48 Endostatin	98 IL-1RI	148 M-CSF	198 Siglec-5	
49 Eotaxin	99 IL-1RIII	149 M-CSF R	199 Siglec-9	
50 Eotaxin-2	100 IL-2	150 MDC	200 ST2 (IL-1R4)	

VI. Troubleshooting guide

Problem	Cause	Recommendation
Weak signal	Inadequate detection	Check laser power and PMT parameters
	Inadequate reagent volumes or improper dilution	Check pipettors and ensure correct preparation
	Short incubation times	Ensure sufficient incubation Time and change sample incubation step to overnight
	Too low protein or antibody concentration in sample	Don't make too low dilution Or concentrate sample
	Improper storage of kit	Store kit at suggested temperature
High background	Excess of protein or antibody	Further dilute protein or antibody
	Excess of streptavidin	Further dilute streptavidin
	Inadequate detection	Check laser power and PMT parameters
	dust	Work in clean environment
	Insufficient wash	Increase wash time and use more wash buffer
Uneven signal	Bubbles formed during incubation	Avoid bubble formation during incubation
	Arrays are not completed Covered by reagent	Completely cover arrays with solution

We also offer customized protein array. You can select the protein of your interest from the following list and we will produce the customized array with affordable price. For more information, please visit our website.

Reference List

Chen,G., Wang,X., Yu,J., Varambally,S., Yu,J., Thomas,D.G., Lin,M.Y., Vishnu,P., Wang,Z., Wang,R., Fielhauer,J., Ghosh,D., Giordano,T.J., Giacherio,D., Chang,A.C., Orringer,M.B., El-Hefnawy,T., Bigbee,W.L., Beer,D.G., and Chinnaiyan,A.M. (2007). Autoantibody profiles reveal ubiquilin 1 as a humoral immune response target in lung adenocarcinoma. *Cancer Res.* 67, 3461-3467.

Huang,R.P. (2003a). Cytokine antibody arrays: a promising tool to identify molecular targets for drug discovery. *Comb. Chem. High Throughput. Screen.* 6, 769-775.

Huang,R.P. (2003b). Protein arrays, an excellent tool in biomedical research. *Front Biosci.* 8, D559-D576.

Zhu,H., Bilgin,M., Bangham,R., Hall,D., Casamayor,A., Bertone,P., Lan,N., Jansen,R., Bidlingmaier,S., Houfek,T., Mitchell,T., Miller,P., Dean,R.A., Gerstein,M., and Snyder,M. (2001). Global analysis of protein activities using proteome chips. *Science* 293, 2101-2105.

Note:

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